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Cardiopulmonary and sedative effects of vatinoxan in sheep receiving atipamezole to reverse medetomidine induced sedation

TIINA KESKITALO

LISENSIAATIN TUTKIELMA

HELSINGIN YLIOPISTO
ELÄINLÄÄKETIETEELLINEN TIEDEKUNTA
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Tekijä - Författare – Author

Tiina Keskitalo

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Tiivistelmä - Referat – Abstract

Medetomidiini on α_2-adrenoseptoriagonisti ja sen käyttö sedatiivina on kliinisessä eläinlääketieteessä hyvin yleistä. Medetomidiinilla saadaan aikaan sedaatio, joka annoksesta ja antoreitistä riippuen vaihtelee kevyestä uneliaisuudesta syvään sedaatioon, joka mahdollista pienet noninvasiiviset toimenpiteet. Medetomidiiniä käytetään usein yhdessä muiden lääkeaineiden kanssa, sillä esimerkiksi medetomidiini ja butorfanoli potentoivat toistensa vaikutuksia. Esilääkityksenä annettu medetomidiini vähentää myös yleisanesteettien tarvetta.

α_2-adrenoseptoriagonistien sedatiivinen vaikutus johtuu keskushermostossa tapahtuvaan presynaptisten α_2-adrenoseptoreiden aktivaatiosta, mikä vähentää noradrenaliinin erittymistä synapsirakoon ja heikentää siten hermoimpulssien kulkua. α_2-adrenoseptoreita on myös keskushermoston ulkopuolella esimerkiksi verisuonten sileän lihaksen pinnalla. Näiden perifeeristen α_2-adrenoseptoreiden aktivaatio lisää verisuonten supistumista.

Medetomidiini aiheuttaa haittavaikutuksina sykkeen ja sydämen minuuttitilavuuden laskua, verisuonten vastuksen nousua, muutoksia verenpaineeseen sekä erityisesti märehtijöillä hapen osapaineen laskua valtimoveressä. Verenpaineen muutos on kaksivaiheinen, sillä verenkierron vastuksen nousu perifeerisestä α_2-adrenoseptoriaktivaatiosta johtuen nostaa verenpaineen ensin korkeaksi. Myöhemmin verenpaine laskee johtuen medetomidiinin sympatolyyttisistä keskushermostovaikutuksista. Verenkierron vastuksen noususta johtuva verenpaineen nousu käynnistää barorefleksin, jonka seurauksena syke laskee entisestään. Medetomidiinin aiheuttama sedaatio voidaan kumota ja haittavaikutuksia lievittää α_2-adrenoseptoriantagonisti atipametsolilla.

Vatinoksaani on toistaiseksi vain tutkimuskäytössä oleva α_2-adrenoseptoriantagonisti, joka voidaan annostella yhdessä medetomidiinin kanssa samassa ruiskussa suonen- tai lihaksensisäisesti. Se ei juuri ylitä veriaivoestettä, eikä siten vaikuta medetomidiinin keskushermostovaikutuksiin, kuten sedaatioon. Se lievittää medetomidiinin aiheuttamia perifeerisestä α_2-aktivaatiosta johtuvaa verenkierron vastuksen nousua ja vähentää siten verenpaineen nousua. Tämä vähentää barorefleksin aktivoitumista ja estää sykkeen ja sydämen minuuttitilavuuden laskua.

Tämän tutkimuksen tavoitteena on selvittää vatinoksaanin, atipametsolin ja medetomidiinin yhteisvaikutuksia, kun vatinoksaani annostellaan lihaksen sisäisesti yhdessä medetomidiinin kanssa. Hypoteesi on, että vatinoksaani lievittää medetomidiinin haittavaikutuksia ilman, että se heikentää rauhoitusta tai heräämistä. Tutkimukseen käytettiin kahdeksaa lammasta, joista kukin sedatoitiin sekä pelkällä medetomidiiniä (30 µg/kg) tai medetomidiini-vatinoksaaniseoksella (30 µg/kg ja 300 µg/kg vastaavasti). 30 minuutin kuluttua annosteltiin atipametsoli (150 µg/kg).120 minuutin seurantajakson aikana kardiopulmonaarisia muuttujia mitattiin tietyin aikavälein. Sedaation tasoa seurattiin subjektiivisesti asteikolla 0-10.

Vatinoksaani lievitti merkittävästi medetomidiinin aiheuttamia muutoksia sykkeeseen atipametsoli-injektion jälkeen ja verenkierron vastukseen jo ennen sitä. Vatinoksaani joudutti verenpaineen nousua ja hapen osapaineen laskua 10 minuuttia medetomidiinin annostelun jälkeen. Sekä sedatoituminen että herääminen oli varmempaa ja nopeampaa vatinoksaanin läsnä ollessa.

Tuloksista voi päätellä, että vatinoksaanin käyttö saattaa parantaa sedaation kardiopulmonaarista laatua ja sen käyttö lampailla on turvallista myös siinä tapauksessa, että sedaatio kumotaan atipametsolilla.

Avainsanat – Nyckelord – Keywords

vatinoksaani, medetomidiini, atipametsoli, sedaatio, lammas

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Johtaja: Outi Vainio

Ohjaajat: Marja Raekallio, Magdy Adam

1 INTRODUCTION

Medetomidine is a highly selective α_2-adrenoceptor agonist, that produces sedation and analgesia in wide range of mammalian species, including ruminants (MacDonald et al. 1988, Mohammad et al. 1993, Bryant et al. 1996). The sedation provided by medetomidine is dose-dependent and varies from mild head drooping to deep sedation enough for minor clinical procedures (Bryant et al. 1996, Romagnoli et al. 2015) The most alarming adverse effects of medetomidine in dogs and horses are bradycardia, decreased cardiac output (CO) and changes in blood pressure (Bryant et al. 1996). In sheep medetomidine also decreases partial pressure of oxygen in arterial plasma (PaO_2), as do also other α-2-agonists (Bryant et al. 1996, Celly et al. 1997).

Sedation, analgesia and reduction of heart rate (HR) caused by medetomidine can be reversed with atipamezole in sheep (Mohammad et al. 1993). Atipamezole is an α_2-adrenoceptor antagonist, which replaces medetomidine in α_2-adrenoceptors in central nervous system and peripheria and causes the animals to gain their feet usually in few minutes, depending on doses and administration route (Ranheim et al. 2000, Granholm et al. 2006).

Vatinoxan, previously known as MK-467 or L-659,066 is a peripheral α_2-antagonist. Because it does not markedly cross the blood brain barrier, it has no direct effects to the sedation (Clineschmidt 1988). In dogs the increase of mean arterial pressure (MAP) and decrease of HR and cardiac index (CI) caused by medetomidine-butorphanol sedation can be partly prevented by simultaneous intravenous (IV) (Salla et al. 2014) and intramuscular (IM) (Salla et al. 2014, Kallio -Kujala et al. 2018) administration of vatinoxan. Similar effect of vatinoxan to HR and MAP was found in horses that were sedated with detomidine, which is another α_2-agonist and causes the same adverse effects as medetomidine (Vainionpää et al. 2013). Raekallio et al. (2010) found out that also in sheep vatinoxan prevents the increase of arterial and venous pressures and the decrease of CO and HR when vatinoxan was administered in same syringe with IV dexmedetomidine.

Medetomidine alone or in combination with ketamine has been used to sedate and immobilize nondomestic ruminants in zoos and in nature. Ketamine is dissociative sedative and it is used in combination with medetomidine to ensure complete immobilization of the animal and safety of the handlers. Medetomidine-ketamine immobilization has been studied for example in free-

ranging impalas (Bush et al. 2004) and in giraffes (Bush et al. 2001), and they also suffer from low PaO_2 with medetomidine doses that led to recumbency (impalas: 220 ± 34 µg/kg medetomidine and 4.4 ± 0.7 mg/kg ketamine, giraffes: 143 -166 µg/cm of shoulder height medetomidine and 27-32 mg/cm of shoulder height ketamine). The sheep in present study may be seen as a model of wild ruminants. Besides getting information about synergy of vatinoxan, medetomidine and atipamezole in sheep, the results provide information that may be applied to other species of small ruminants in zoos and in wilderness.

Even though it is known, that vatinoxan has beneficial effects when given with α_2-adrenoceptor agonists, it remains still unclear how vatinoxan and atipamezole affect to each other in sheep when the sedation is induced by medetomidine. The aim of this study was to reveal effects of vatinoxan to sedation, reversal and cardiopulmonary parameters to sheep that were first sedated with medetomidine and vatinoxan in same syringe and after 30 minutes reversed with atipamezole. Our hypothesis was that presence of vatinoxan leads to better cardiopulmonary quality of the sedation and to faster recovery. Data used in this study is part of wider data collected in 2016 and the results have been previously published by Adam et al. (2018a).

2 LITERATURE REVIEW

2.1 Cardiopulmonary quality of sedation

Delivering oxygen to tissues and carrying by-products of cellular metabolia and foreign substances to the appropriate organs to be eliminated are the main functions of blood circulation in short term consideration (Muir 2007). In longer consideration, functions of blood circulation are to carry nutrients, defend body against foreign substances with white blood cells and to prevent hemorrhage by clotting (Muir 2007). Organs mainly responsive of oxygen delivery and other functions of blood are the heart, blood vessels, lymph vessels and lungs, but also gastrointestinal system, liver and kidneys contribute to maintaining homeostasis in body (Muir 2007).

Cardiopulmonary status of an animal under sedation or general anesthesia is easily altered because anesthetic drugs affect to mechanisms that control the circulatory system. Cardiopulmonary status can be assessed by measuring and monitoring several parameters. The main concern during a short-term clinical sedation or anesthesia is oxygen delivery to tissues (Muir 2007). It cannot be measured directly, but it can be evaluated and calculated by monitoring other parameters, such as HR, CO, PaO_2 and hemoglobin concentration (Hb) (Boyd et al. 1991).

2.1.1 Physiological control mechanisms of the circulatory system

Cardiopulmonary system is regulated by combined effects of the central and peripheral nervous system (neural or autonomic control), vasoactive substances in circulating blood (humoral control) and tissue mediators that locally modulate vascular tone (local control) (Muir 2007, Heerdt & Crystal 2013). Neural control of circulatory system consists of autonomous reflex arches, which are triggered by information gathered from inside or outside of the body. Information is processed in central nervous system (CNS) and appropriate output is delivered to heart and vessels via efferent nerve fibers. Information to induce these reflex arches includes sensory data of the circumstances outside the body and information of the blood flow and content gathered by peripheral baroreceptors (blood pressure), mechanoreceptors (volume) and chemoreceptors (gas tensions). In brain this information results in modulatory responses, which are carried by efferent

sympathetic or parasympathetic nerves to make appropriate adjustments to the function of heart and blood vessels (Muir 2007).

Humoral control of the circulatory system is mediated by vasopressin released from posterior pituitary and by vasoactive catecholamines adrenaline and noradrenaline, which are released to blood from adrenal medulla (Muir 2007). The release of catecholamines is induced by pain, trauma, hypotension, hypothermia and stress or fear (Muir 2007). The adrenal medulla is innervated by preganglionic parasympathetic nerve fiber, but instead of sending axons to target organs, it releases catecholamines in to the circulation (Muir 2007). Increase in circulating adrenalin and noradrenalin leads to increased heart and respiratory rates, cardiac contractility and redistribution of blood flow by dilating vascular beds in skeletal and cardiac muscle and constricting arteolies in visceral organs and skin (Muir 2007).

Vasopressin is released by increased plasma solute to conserve water in collecting ducts of kidney and thus return the plasma osmolality and volume back to normal (Muir 2007). Vasopressin acts as a vasoconstrictor specially in mesenteric vessels and thus redistributes the systemic blood flow. Release of vasopressin occurs in pain, stress, hypoxia, and in response to increased plasma solute (Muir 2007). Thereby humoral control of the circulatory system is complementary to sympathetic nerve stimulation and together humoral and neural control systems provide both rapid (neural) and prolonged (humoral) responses to stressful situations (Muir 2007).

Tissues can regulate their own blood flow during chances of circumstances to keep the oxygen tension adequate (local control). Local vasodilatory mediators released from vascular endothelial cells dilate small arterioles when the oxygen tension is decreased, or vessel walls stretch again after an occlusion (Muir 2007).

2.2 Cardiopulmonary variables

As mentioned earlier, several sedatives and general anesthetics effect to the physiological control mechanisms of cardiopulmonary system. Under sedation or general anesthesia cardiopulmonary function is monitored with devices that measure parameters that represent cardiopulmonary status.

2.2.1 Heart rate

HR is directly regulated by neural and humoral control systems. Neural ways to control HR include both sympathetic and parasympathetic stimulation (Muir 2007). Sympathetic activation is mediated by noradrenaline released from postganglionic sympathetic nerve fibers (Muir 2007). In the heart, sympathetic nerve fibers end up in sinoatrial node, atrioventricular node, atria and myocardium and the released noradrenalin binds to β_1- and β_2-adrenoceptors on cardiac cell membranes (Muir 2007). Activation of β_1- and β_2-adrenoceptors induces a G-protein mediated response in cardiac cells that leads to increased HR and myocardial contractility (Muir 2007). Humoral control of the HR is mediated by both adrenalin and noradrenalin and is complementary to the sympathetic activation (Muir 2007).

Parasympathetic stimulation to the heart is delivered via the vagal nerve and mediated by acetylcholine at synapses of parasympathetic ganglia and neuromuscular junctions at membranes of cardiac cells. In heart the vagal nerve innervates the sinoatrial node, the atrioventricular node and the atrial myocardium. In the cardiac neuromuscular junctions acetylcholine binds to muscarinic receptors and activates G-protein mediated chain that leads to decreased HR and cardiac contractility (Muir 2007). Baroreflex is a reflex arch, that consist of information of increased arterial blood pressure from carotid baroreceptor and by central processing leads to parasympathetic output that lowers HR, myocardial contractility and peripheral vasoconstriction (Benarroch 2008).

There are several methods to assess the HR of animal. In medetomidine related studies HR was measured directly from the sheep by auscultating with a stethoscope (Mohammad et al. 1993, Kästner 2006), by a chart recorder via arterial catheter (Bryant el al. 1996) or by electrocardiography (Celly et al. 1997).

2.2.2 Cardiac output

CO is the volume of blood pumped by heart in a minute. It consists of HR and stroke volume, which depends on heart muscle contractility. CO is controlled by neural and humoral control mechanisms simultaneously with HR. Autonomous sympathetic and parasympathetic stimulations affect to both HR and stroke volume (Muir 2007). CO can be altered by matters outside the physiological control mechanisms. For example, poor venous return, low end-diastolic ventricular filling and regurgitating atrioventricular valves lead to reduced CO (Haskins 2007). Increased afterload due to increased systemic vascular resistance (SVR) also decreases CO (Haskins 2007)

Golden standard for measuring the CO is the aortic flow probe, but because of its extreme invasiveness (thoracotomy) it is rarely used (Flammer et al. 2013). Of more indirect methods lithium dilution (LiDCO) has been shown to be suitable and convenient way to measure CO (Kurita et al. 1997, Linton et al. 2000, Flammer et al. 2013). In lithium dilution method a designated amount of isotonic lithium is delivered to central vein as a bolus and the change in lithium concentration is monitored with a sensor in carotid artery, and the result is computed from the concentration data combined with waveform data of arterial pressure (Flammer et al. 2013).

Other ways to measure CO are dye dilution, thermodilution, transesophageal Doppler echocardiographic, thoracic electrical bioimpedance and pulse analysis (Haskins 2007). Dye and thermodilution methods are based on the same principle of injecting substance (color or iced saline or dextrose) to central vein and monitoring it from the arterial side, but in these methods from pulmonary artery via special thermodilution catheter slided to pulmonary artery from jugular vein instead of monitoring the change from carotid artery as in LiDCO-system (Linton et al. 2000, Haskins 2007).

CO of sheep in medetomidine related studies has been measured with lithium dilution (Rekallio et al. 2010), dye dilution (Bryant et al. 1997) and thermodilution (Kästner et al. 2007, Talke et al. 2000). CO is not regularly measured during clinical anesthesia or sedation and normal values of sheep cannot be found in the available literature. Cardiac index (CI) is CO divided by the weight of

the subject and its unit is L/min/kg. In this manner values of various sized animals are more comparable with each other.

2.2.3 Blood pressure

Elasticity and the amount of smooth muscle and fibrous tissue differ in vessel walls in a manner, that enables appropriate pressure and blood flow in different sized vessels. Arterial walls are thicker and more elastic than venous walls (Muir 2007). Pressure in left atrium, ventricle and aorta varies in a waveform manner depending on the contractility of cardiac muscle cells (Muir 2007). In arteries, arterioles and capillaries pressure decreases dramatically and is lowest in venules. In central vein pressure increases again, yet not nearly as high as in arterial side (Muir 2007). Pressure curves in right atrium and ventricle are waveform (Muir 2007). The contractility of right side of the heart is weaker compared to left side, which leads to much lower blood pressure in pulmonary circulation than in systemic circulation (Muir 2007).

Peak value of arterial pressure wave is referred as systolic arterial pressure (SAP) and the valley value as diastolic arterial pressure (DAP). MAP is estimated from SAP and DAP by the formula (Muir 2007):

$$MAP = DAP + \frac{SAP - DAP}{3}$$

Arterial pressure depends on HR, stroke volume, vascular resistance, arterial compliance, and blood volume. These variables are controlled neurally and humorally and are altered by many anesthetic drugs. Normal SAP in sheep is 80-100 mmHg, DAP 60-80 mmHg and MAP 75-100 mmHg (Lin et al. 2012). In mammalian species hypotension is defined as MAP below 65 mmHg (Clarke 2014). Untreated prolonged hypotension during anesthesia may lead to cardiac arrest, neurological deficits, blindness or renal failure (Clarke 2014)

In medetomidine related studies arterial pressure is measured invasively from the sheep using pressure transducer attached to arterial catheter and placed to level of the heart base (Kästner 2006, Bryant et al. 1996, Celly et al. 1997). Central venous pressure (CVP) has been measured

using pressure transducers attached to central venous catheter inserted from left jugular vein (Raekallio et al. 2010, Adam et al. 2018b).

2.2.4 Systemic vascular resistance

SVR is a quantity that is calculated to represent vascular tone and afterload of the heart (Muir 2007). Vascular tone is altered by local vasodilating or vasoconstrictive substances released from tissue or vessel wall and by circulating vasoactive catecholamines (Muir 2007).

SVR is calculated with the following equation (Muir 2007):

$$SVR = \frac{80 * (MAP - CVP)}{CO}$$

2.2.5 Partial pressure of oxygen in plasma

In lungs oxygen passes through the alveolar and capillary membranes. Normally the total distance across the membranes is less than 1 µm, and the partial pressure of oxygen in plasma of arterial capillaries in the lungs nearly equals the partial pressure of oxygen in the alveoli so that the normal values of PaO_2 range between 80 and 110 mmHg when breathing room air in normal barometric pressure at sea level (Haskins 2007). Animals with PaO_2 lower than 80 mmHg are considered hypoxemic and a value lower than 60 mmHg under anesthesia should be a trigger for symptomatic therapy (Haskins 2007).

Due to hemoglobin the total amount of oxygen in blood is about 60 times greater than the amount dissolved in plasma under the normal circumstances (Haskins 2007). PaO_2 measures only dissolved oxygen and does not consider the oxygen bound into hemoglobin (Haskins 2007).

PaO_2 measures the ability of the lung to deliver oxygen to the blood (Haskins 2007). Anesthetic drugs generally cause the partial pressure of oxygen to decrease because of hypoventilation,

increased ventilation-perfusion mismatching, and atelectasis caused by recumbency (Haskins 2007). Partial pressure of oxygen in plasma is measured from arterial or venous blood directly with blood gas analyzer.

2.2.6 Oxygen delivery

Oxygen delivery (DO_2) is the volume of oxygen delivered to tissues in a minute. It depends on total oxygen content of blood and CO (Boyd et al. 1991). Under normal circumstances most of the oxygen in blood is bound by hemoglobin and only a small fraction is dissociated in plasma. When oxygen is delivered to tissues from plasma, the balance is achieved again by dissociation of more oxygen from hemoglobin.

Relative amount of oxygen bound by hemoglobin depends on the amount of dissolved oxygen in plasma. The oxyhemoglobin dissociation curve as the function of partial pressure of oxygen in plasma is sigmoid shaped, which means that hemoglobin is nearly fully saturated even when the PaO_2 has decreased to 80 mmHg (Haskins 2007). Marked change in hemoglobin oxygen saturation occurs between 20 and 60 mmHg of PaO_2 and at that level amount of oxygen bound to hemoglobin decreases dramatically (Haskins 2007). Interstitial fluid normally has the partial pressure of oxygen 30 mmHg, so the oxygen is effectively transferred from hemoglobin of the blood into the tissues (Haskins 2007).

In adult sheep there are two genetically different types of hemoglobin, A and B (Maginnis et al. 1986). They are controlled by two autosomal alleles with codominant expression (AA, AB, BB) (Cohen et al, 1956). Type A has greater affinity to oxygen than type B, which means that type A is more saturated in constant partial pressure of oxygen and their oxyhemoglobin dissociation curves differ from each other (Maginnis et al. 1986). In pH 7.5 hemoglobin type AA has P_{50} in partial pressure of oxygen 31.3 mmHg, type BB in partial pressure of oxygen 40.7 mmHg and the heterozygote type AB in partial pressure of oxygen 35.7 mmHg (Maginnis et al. 1986).

Equations to hemoglobin saturation with oxygen (SO_2) in sheep for hemoglobin types AA, AB and BB by Maginnis et al. (1986) are respectively:

AA: $SO_2 = (\frac{25.290}{P^3 - 10{,}3P^2 + 152P} + 1)^{-1}$

AB: $SO_2 = (\frac{41.460}{P^3 - 9.0P^2 + 214P} + 1)^{-1}$

BB: $SO_2 = (\frac{60.900}{P^3 - 10.9P^2 + 280P} + 1)^{-1}$

Mixed AA + BB: $SO_2 = (\frac{45.460}{P^3 - 5.8P^2 + 175P} + 1)^{-1}$

Oxygen content of arterial blood (CaO_2) is calculated from measured Hb, PaO_2 and calculated SO_2 with equation:

$$CaO_2\ (\mathrm{ml/l}) = [(1{,}39 \times \mathrm{Hb} \times SO_2) + (0.0031 \times PaO_2)] \times 10$$ (Boyd et al. 1991)

DO_2 is a variable that is calculated by using the measured CO and CaO_2:

$$DO_2\ (\mathrm{ml/min}) = \mathrm{CO} \times CaO_2$$ (Boyd et al. 1991)

Oxygen delivery index (DO_2I) is DO_2 divided by the bodyweight of the subject for better comparability between various sized animals.

2.3 Medetomidine

Medetomidine, first introduced as sedative by Savola et al. (1986), is an alfa-2-adrenoseptor agonist that is used in veterinary medicine to provide sedation and analgesia for minor noninvasive medical procedures and used as premedication before general anesthesia. In Finland medetomidine is licensed for use in dogs and cats. Since maximum residual limit (MRL) is not defined, it is not allowed to be used in food producing animals. Medetomidine is a racemic mixture of stereoisomers dexmedetomidine and levomedetomidine, of which dexmedetomidine is

the active enantiomer as levomedetomidine has no sedative nor analgesic effect (MacDonald et al. 1991).

Medetomidine is known to have a dose-dependent sedative effect on wide range of mammalian species including at least rat (MacDonald 1988), ponies (Bryant et al. 1991) and sheep (Mohammad et al. 1993), dog (Vähä-Vahe 1989), cat (Vähä-Vahe 1989), human (Kauppila 1991) and several wild ruminants (Jalanka 1989, Bush et al. 2004) (table 1).

2.3.1 Pharmacokinetics of medetomidine in sheep

Elimination half-life of medetomidine (single dose 15 µg/kg IV) in sheep was 37.85 ± 2.84 minutes (Muge et al 1996). Volume of distribution is 2.69 ± 0.62 l/kg (Muge et al. 1996), which means that it spreads effectively from blood to tissues. Terminal half-life of medetomidine (30 µg/kg IM) was 32.7 ± 14.9 min and the volume of distribution 3.9 ± 2.4 l/kg (Kästner et al. 2003). Clinical sedative effect correlated to plasma concentration, which in IM administration has its peak after 30-40 minutes and in IV administration few minutes after delivery (Muge et al. 1996, Kästner et al. 2003).

2.3.2 Mechanism of sedative and analgesic effect

Medetomidine, or more precisely its active enantiomere dexmedetomidine effects as an agonist mainly to adrenergic α_2-receptors (Savola et al. 1986, McDonald et al. 1991). α_2-adrenoceptors are found pre- and postsynaptically (Ebert 2013, Lemke 2007). Physiological function of presynaptic adrenoceptors is to autoregulate the release of noradrenalin to the synapse by a negative feedback loop after an adequate amount of noradrenalin is released to synapse to transmit the neural activity (Ebert 2013). Postynaptic α_2-adrenoceptors are found on the surface of blood vessels, platelets and several tissues including liver, pancreas and kidney, and they contribute to redistribution of blood flow when noradrenalin or adrenalin is released during sympathetic activity (Ebert 2013).

Sedative effect of medetomidine and other α_2-adrenoceptor agonists is mediated in central nervous system by α_2-adrenoceptors, that locate presynaptically on noradrenergic neurons in *locus coeruleus* (Lemke 2007). Reduction of sympathetic outflow results of activation of α_2-adrenoceptors in efferent neurons in medullary dorsal horn area (Lemke 2007). α_2-adrenoceptors co-located with opioid receptors in dorsal horn of the spinal cord modulate the afferent pain signal and mediate the analgesic effect of α-adrenoceptor agonists (Ebert 2013).

2.3.3 Doses and sedative effect of medetomidine

Medetomidine can be administered subcutaneously (SC), IM or IV depending on the wanted onset time of the sedation. Fastest onset is achieved by IV and slowest by SC administration. Recommended dose range in summary of product characteristics of medetomidine for dogs is 10-80 µg/kg (Domitor, Orion Pharma). Low doses of IM medetomidine (5 µg/kg and 10 µg/kg) have been studied in sheep in purpose to reveal its efficacy as premedication for general anesthesia (Kästner 2006). Lower dose (5 µg/kg) produced light sedation without ataxia or loss of spontaneous activity and the higher dose (10 µg/kg) made the sheep mildly atactic yet standing and caused them to lose spontaneous activity (Kästner 2006). Both doses proved to lower the doses of propofol needed for induction 30 minutes after medetomidine administration and isoflurane needed to maintain general anesthesia during an experimental orthopedic surgery (Kästner 2006). 30 µg/kg IM produced sedation with lateral recumbency before induction (Kästner 2006).

Medetomidine (40 µg/kg IM) produces sedation and recumbency for 1 hour and good analgesia against pinprick for 30-45 minutes in sheep (Mohammad 1993). Constant rate infusion of 3 µg/kg/h medetomidine intraperitoneally to adult sheep is known to provide statistically significant analgesia in acute post-surgical pain with no sedative effect (Murdoch 2013).

2.4 Cardiopulmonary adverse effects of medetomidine in sheep

Vasoconstriction, alterations in blood pressure and bradycardia are the main cardiovascular adverse effects of medetomidine in all mammalian species (Clarke 2014). Pulmonary adverse

effects vary more between species, and ruminants are more predisposed to have difficulties in ventilation and decrease in PaO_2 than dogs and cats (Clarke 2014).

Cardiopulmonary adverse effects of medetomidine are mediated by both central and peripheral α_2-adrenoceptor activation (Clarke 2014). Activation of α_2-adrenoceptors located peripherally on surface of blood vessel blood muscle causes vasoconstriction and increases SVR and MAP (Clarke 2014). Increase of MAP activates the baroreflex, which decreases HR (Muir 2007, Benorrach 2008). Decreased HR and increased SVR decrease the CO and DO_2 is compromised (Muir 2007). Sedation itself and suppressive effect to central cardiovascular centre of medetomidine is believed to play also a major role in decrease of HR (Clarke 2014).

2.4.1 Heart rate

In sheep, medetomidine (5, 10 and 20 µg/kg IV) significantly decreased HR compared to baseline values (Bryant et al. 1996). HR decreased from 70-90 beats/min initially to 30-50 beats/min for a couple of minutes but increased to 45-60 after 5 minutes (Bryant et al 1996). The effect lasted over 60 minutes and it was not dose related (Bryant et al. 1996). Similar results were detected by Celly et al (1997), who reported that 10 µg/kg medetomidine IV in sheep decreased HR significantly 2 minutes after administration (40.3 ± 6.4 beats/min) compared to placebo treatment (80.6 ± 4.8) (Celly et al. 1997). HR in medetomidine treatment tended to stay below placebo treatment values at least for 60 minutes after administration, but after 2 minutes difference was no longer significant (Celly et al. 1997). When the sheep calmed down, the HR decreased from the baseline values also in the saline treatment and HR did not differentiate between medetomidine and saline treatments after 30 minutes (Bryant et al. 1996).

Medetomidine (5 and 10 µg/kg IM) significantly decreased HR compared to saline control group in sheep (Kästner et al. 2006). General anesthesia was induced with propofol 30 minutes after medetomidine or saline injection and maintained with isoflurane during an experimental orthopaedic surgery. In that study HR of the sheep in both medetomidine groups (5 and 10 µg/kg)

stayed significantly below the HR of the control group for the whole follow up period of 90 minutes (Kästner et al. 2006).

In sheep medetomidine (40 µg/kg IM) decreased HR significantly 15, 45 and 75 minutes after administration (52,7 ± 5, 58 ± 10 and 51,3 ± 2,6 beats/min respectively) compared to baseline level (82,4 ± 6,2 beats/min) (Mohammad 1993). There was no control group in this study, which may not be necessary when values of sedated animals are compared to baseline, but as study by Bryant et al. (1996) showed, HR may decrease also in control group.

Mentioned studies show that medetomidine significantly decreases HR and IV route of medetomidine results more rapid and profound decrease compared to IM administration (Mohammad 1993, Bryant et al. 1996, Celly et al. 1997, Kästner et al. 2006).

2.4.2 Arterial blood pressure

In sheep medetomidine increased MAP significantly from baseline values within a few minutes after IV administration (Bryant el al. 1996, Raekallio et al. 2010). After an initial increase, MAP tended to decrease below the baseline level, but the change was not significant in studies by Bryant et al. (1996) or Raekallio et al. (2010). However, Celly et al (1997) detected a significant decrease of MAP at 45 minutes after drug administration compared to placebo treatment. Biphasic changes in MAP are also reported by Talke et al. (2000). After IM administration initial increase in MAP was not as notable as with IV administration (Celly et al. 1997, Kästner 2006).

Biphasic changes in MAP are explained similarly to changes in HR. Initial increase of MAP is a consequence of vasoconstriction and increased SVR induced by medetomidine (Lemke 2007). Latter prolonged phase of decreased MAP is a consequence of central sympatholysis (Clarke 2014).

2.4.3 Pulmonary adverse effects

Dexmedetomidine (2 µg/kg IV) caused pulmonary oedema seen as changes in density in CT to sheep (Kästner et al 2007). Changes were most profound at 9-12 minutes after injection and signs of regression of the oedema were seen at 30 minutes after injection. Alterations in CT co-occurred with dramatic decrease of PaO_2 and increase in pulmonary blood pressure (Kästner et al. 2007)

2.5 Atipamezole in sheep

Atipamezole is a specific α-2-adrenoceptor antagonist that reverses the sedative effect of medetomidine and mitigates majority of the adverse effects (Virtanen 1989, Clarke 2014). With IM administration atipamezole reversed sedative effect caused by IM medetomidine and helps dogs to gain their feet in 4-12 min (Vainio & Vähä-Vahe 1990), and alleviated medetomidine-induced cardiopulmonary adverse effects (Vainio 1990).

In Finland atipamezole is licensed for dogs and cats. Since MRL is not defined, it is not allowed to be used in food producing animals According to the summary of the product characteristics dose of atipamezole is in dog 5 times greater and in cat 2,5 times greater than preceding dose of medetomidine. Vainio and Vähä-Vahe (1990) suggested doses four, six or ten times higher than preceding dose of medetomidine for dogs. In their study atipamezole was injected IM 20 minutes after medetomidine. There was no excitement or over-alertness after atipamezole, but 41% of the dogs became drowsy 0,5 – 1 h after atipamezole injection (Vainio & Vähä-Vahe 1990). Drowsiness after initial reversal, or resedation after atipamezole is also documented in reindeer (Ranheim et al. 1997). Ranheim et al (1997) suggested that resedation may be explained by longer elimination half-life of medetomidine compared to atipamezole.

Atipamezole has been studied in sheep to reverse the sedation and cardiopulmonary adverse effects of mere medetomidine (Talke et al. 2000) and medetomidine-ketamine (Caulkett et al. 1994). Atipamezole (a 5-minute IV constant rate infusion (CRI) 2.5 times greater than medetomidine) reversed the sedation and changes in SVR, MAP CO and HR induced by

medetomidine (IV to target plasma levels of 0.8, 1.6, 3.2, 6.4, and 12.8 ng/ml) in sheep (Talke et al. 2000). Caulkett et al. (1994) showed that atipamezole (625 µg/kg IV) reversed the severe hypoxemia caused by medetomidine-ketamine (125 µg/kg IV and 2.5 µg/kg IV respectively).

2.5.1 Pharmcokinetics of atipamezole and its effect to pharmacokinetics of medetomidine

When atipamezole was administered to sheep 200 µg/kg IV 60 minutes after medetomidine (40 µg/kg IV) its elimination half-life was 34.2 ± 11.9 minutes and volume of distribution 2.0 ± 0.54 l/kg (Ranheim et al. 2000).

Atipamezole administered after medetomidine alters also the pharmacokinetics of medetomidine (Ranheim et. al 1997, 2000). When atipamezole (200 µg/kg IV) was administered to sheep 60 minutes after medetomidine (40 µg/kg IV), elimination half-life of medetomidine shortened significantly compared to mere medetomidine without atipamezole (21.8 (5.2) min and 34.8 (7.3) min respectively) (Ranheim et al. 2000). In semi-domesticated reindeer atipamezole (300 µg/kg IV) 60 minutes after medetomidine (60 µg/kg IV) resulted in 2.5-3.5-fold increase in medetomidine plasma concentration and increased medetomidine clearance significantly (Ranheim 1997).

Talke et al. (2000) assessed organ blood flow after medetomidine infusion to target plasma levels with and without subsequent 5-minute CRI atipamezole 2,5 times greater than medetomidine infusion in sheep. They noticed, that medetomidine IV to target plasma levels of 0.8, 1.6, 3.2, 6.4, and 12.8 ng/ml initially decreased blood flow to skeletal muscle, cerebral cortex, skin and distal ileum, but after atipamezole blood flow returned closer to baseline values. Atipamezole reverses the medetomidine induced redistribution of blood flow and thus accelerates the clearance of medetomidine (Talke et al. 2000).

2.6 Vatinoxan in sheep

Vatinoxan is a α_2-adrenoceptor antagonist that poorly penetrates to central nervous tissue (Clineschmidt et al. 1988). It is in experimental use and it is not licensed in veterinary use. In sheep vatinoxan attenuated changes in MAP, SVR, CO and HR induced by dexmedetomidine (Raekallio et

al. 2010). Vatinoxan has been tested with various doses (150 µg/kg, 300 µg/kg or 600 µg/kg IM) on sheep receiving atipamezole (150 µg/kg, IM) to reverse medetomidine-ketamine (30 µg/kg and 1 mg/kg respectively IM) induced sedation (Adam et al. 2018b). Prior to the reverse all tested doses of vatinoxan alleviated medetomidine-ketamine induced changes in cardiovascular variables, but the effect was most profound with 600 µg/kg of vatinoxan (Adam et al. 2018b). With vatinoxan HR and CVP recovered to baseline level after initial changes and SVR did not increase significantly from baseline at all (Adam et al. 2018b). After reverse there was no significant differences between treatments in cardiopulmonary variables, except in HR, which was higher with 600 µg/kg vatinoxan compared to other treatments (Adam et al. 2018b). Vatinoxan (150 µg/kg IV) has been reported to alleviate pulmonary oedema, bronchoconstriction and hypoxaemia induced by dexmedetomidine in sevoflurane anesthetized sheep when administered 10 minutes prior to medetomidine (3 µg/kg IV) (Adam et al. 2018c).

Vationoxan (150, 300, and 600 µg/kg IM) alleviated medetomidine-ketamine (30 and 1 µg/kg IM respectively) induced decrease in HR and PaO_2 and increase in MAP, CVP and SVR with no effect to reversal with atipamezole (150 µg/kg IM) 60 minutes after in sheep (Adam et al. 2018b). After the atipamezole administration at 60 minutes, there was no significant difference in cardiopulmonary variables whether vatinoxan was present or not (Adam et al. 2018b).

3 MATERIALS AND METHODS

3.1 Animals and instrumentation

Eight ewes, five crossbreed and three Texel, were used in this study. The sheep were 1-5 years old and weighted 44-78 kg. They were considered healthy based on physical examination, serum chemistry analysis and complete blood counts. Right carotid arteries of the sheep were surgically elevated into subcutaneous space six months before the beginning of the study. Thereafter the sheep had been used in another vatinoxan related study, in which they were sedated with medetomidine and ketamine (Adam et al. 2018b). Sheep were housed in one group in the Laboratory Animal Centre of the University of Helsinki. They were fed twice a day with hay, minerals and oat or commercial concentration and they had free access to water. In the study day morning sheep were fed only with hay and free water. The study was approved by the National Animal Experiment Board (ESAVI/9394/04.10.07/2015).

During the study sheep were hanging in a sling so that their claws are 2-5 cm above the ground. Their feet were set through the sling and weight was on the abdomen and sternum (figure 1.). The sheep stayed in standing position during the sedation and their heads were kept in natural position to allow them to breath as freely as possible and the saliva to drip out from the mouth to avoid aspiration of saliva.

Figure 1. *A sheep hanging in the sling ready to be instrumented.*

Blood pressures and HR were recorded (S/5 Compact Critical Care Monitor, Datex-Ohmeda; GE Healthcare, Finland) at baseline and at intervals to 120 minutes after treatment. CVP was measured using a 40 cm long catheter (Cavafix Certo; B. Braun Melsungen, Germany), which was placed into *Vena cava* through left *Vena jugularis*. Arterial pressures (systolic, diastolic and mean) and the HR were measured from a catheter (20-gauge arterial catheter, Becton Dickinson, Sandy, Utah, USA) in the elevated right carotid arteria. Both catheters were equipped with three way valves, and the blood samples for pO2 and pCO2 analyzes were taken with 2 ml blood gas syringes (Pico50; Radiometer, Copenhagen, Denmark). Full blood gas syringes were stored in ice box and analyzed (GEM Premier 4000, Bedford, Massachusetts, USA) within 15 minutes from drawing the sample. Body temperature was measured right before each blood sample, and the results were corrected to the temperature. Concentrations of sodium and hemoglobin are analyzed from the arterial samples simultaneously with the blood gases and used for correcting the CO.

CO was measured with lithium dilution method (LidCO plus Hemodynamic Monitor; LidCO Ltd, Cambridge, UK) at baseline and at 20, 40, 50, 60, 75 and 90 min after treatment. Lithium chloride (0.3 mmol of lithium chloride 0.15 mmol／mL, LidCo Ltd) was injected as a bolus and flushed with 20 ml of saline through a catheter (18-gauge venous cannula, Terumo Europe, Leuven, Belgiumin)

in right jugular vein. LiDCO-sensor (CM10) and LiDCO Flow regulator were placed on the catheter in the right *Arteria carotis*. Sensor was chanced daily, so that the CO follow up of two individual sheep were measured with one sensor. Standard values of 10 g/dL of hemoglobin and 140 mmol/L of sodium were used in LiDCO settings during the measurements and the CO values were later corrected using the hemoglobin and sodium values obtained simultaneously with the arterial blood gas analysis. Sedation was scored visually on scale 0-10, where 0 means no sedation and 10 means deep sedation with no response to manipulation or hand clapping.

3.2 Treatments and the experiment protocol

Each of the eight ewes were given two treatments with minimum of 14 days washout period between treatments. Both treatments included sedation with medetomidine HCl (Dorbene 1 mg/ml; Syva Laboratories S.A., Spain) (30 µg/kg IM) at timepoint zero. Sheep got either mere medetomidine or medetomidine and vatinoxan HCl (Vetcare Ltd., Finland) (300 µg/kg IM) (MV + ATI) dissolved in the same syringe. 30 minutes after the sheep were given atipamezole (Alzane 5 mg/ml; Syva Laboratories) (150 µg/kg IM) in both treatments. The medication was injected to semimembranosus muscle, and the researcher administering the medication and recording the results was not aware of the content of the syringes.

3.3 Statistical analysis

Generalised linear mixed model was used to assess the statistical differences between treatments within each timepoint for HR, MAP, CI, SRV, PaO_2 and DO_2I. P-value lower than 0.05 was considered as statistically significant.

4 RESULTS

4.1 Cadiovascular variables

Data of HR and MAP are presented in figure 2 and CI and MAP in figure 3 as mean ± SD. HR decreased initially in both treatments. It tended to decrease faster with vatinoxan, but the difference between treatments was not significant before administration of atipamezole. HR was significantly higher with vatinoxan at 90 minutes and later. Cardiac index did not differ significantly between treatments. MAP increased initially faster with vatinoxan than without it, but there was no significant difference between treatments at time points after 10 minutes. SVR increased without vatinoxan and was significantly higher at timepoints 20 and 50 minutes compared to treatment with vatinoxan.

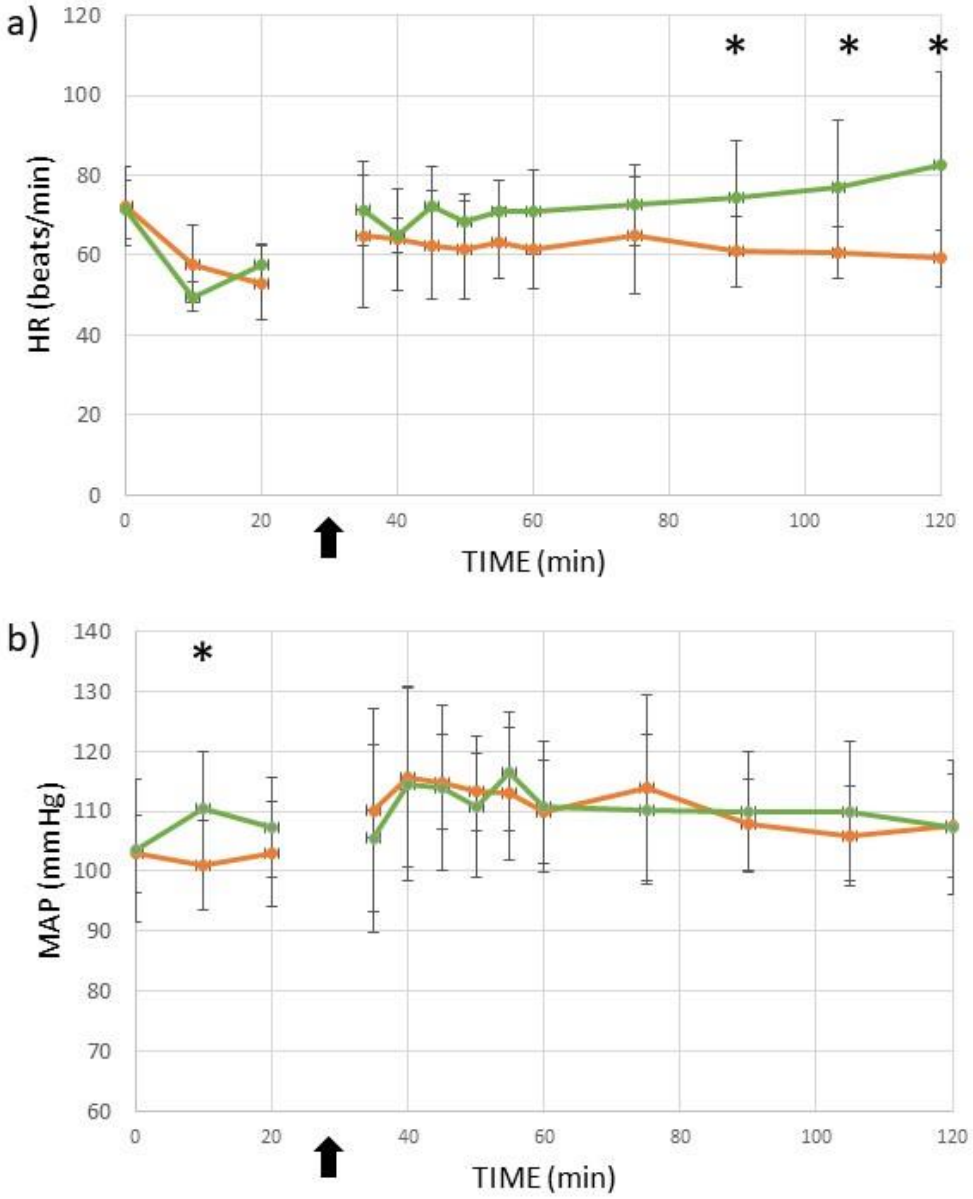

Figure 2. Mean ± SD of HR (a) and MAP (b) in eight sheep treated with medetomidine (30 µg/kg IM) (MED + ATI, orange line) or medetomidine (30 µg/kg IM) and vatinoxan (300 µg/kg IM) (MV + ATI, green line) in same syringe. Sedation was reversed with atipamezole (15 µg/kg IM) after 30 minutes in both groups (black arrow). Statistically significant ($p < 0.05$ difference between treatments are indicated by *.

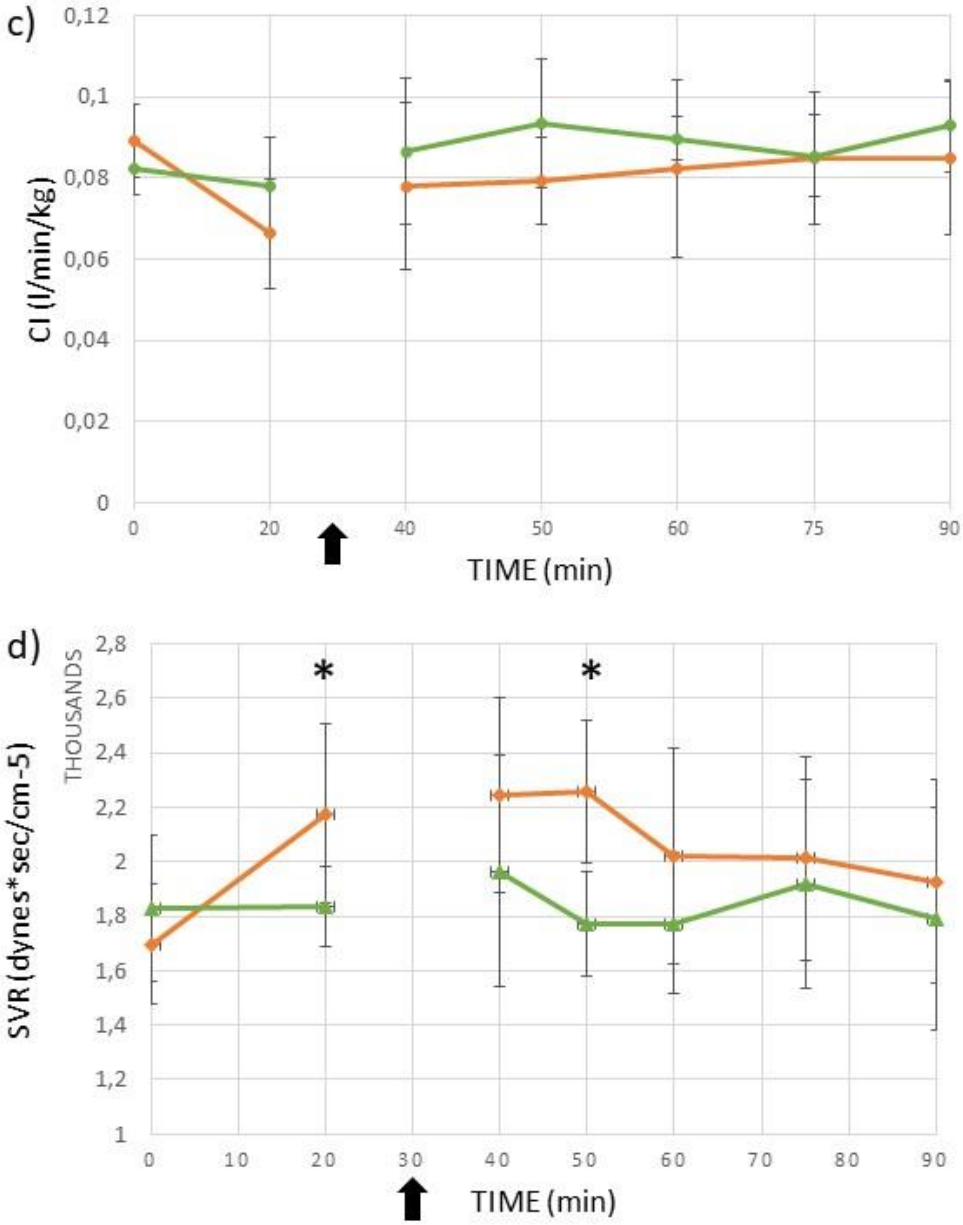

Figure 3. Mean ± SD of CI (c) and CVR (d) in eight sheep treated with medetomidine (30 µg/kg IM) (MED + ATI, orange line) or medetomidine (30 µg/kg IM) and vatinoxan (300 µg/kg IM) (MV + ATI, green line) in same syringe. Sedation was reversed with atipamezole (15 µg/kg IM) after 30 minutes in both treatments (black arrow). Statistically significant ($p < 0.05$ difference between treatments are indicated by *.

4.2 Pulmonary variables

Data of PaO_2 and oxygen delivery index are presented in fig(4) as mean ± SD. PaO_2 decreased initially more and faster in MV + ATI group compared to MED + ATI group. After 10 minutes there

was no significant difference between treatments in PaO_2. In DO_2I there was no significant difference between treatments.

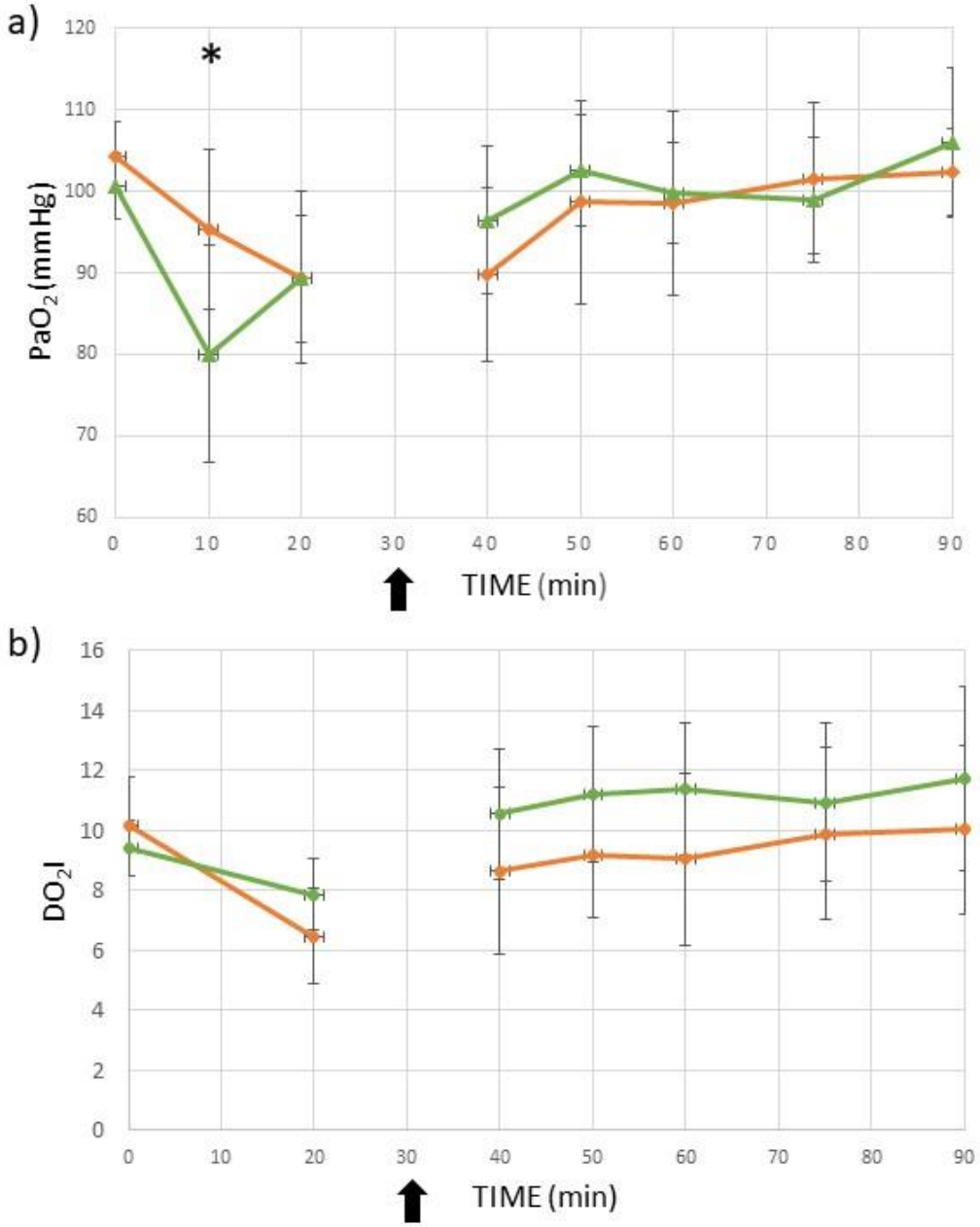

Figure 4. Mean ± SD of PaO_2 (a) and DO_2I (b) in eight sheep treated with medetomidine (30 µg/kg IM) (MED + ATI, orange line) or medetomidine (30 µg/kg IM) and vatinoxan (300 µg/kg IM) (MV + ATI, green line) in same syringe. Sedation was reversed with atipamezole (15 µg/kg IM) after 30 minutes in both treatments (black arrow). Statistically significant ($p < 0.05$) difference between treatments are indicated by *.

4.3 Sedation score

Sedation scores of each sheep in presented in figure 5. In both groups some degree of sedation was achieved before atipamezole administration, but in vatinoxan group onset of sedation was faster and more profound. Resedation, defined as increase of three or more grades after initial reverse, happened in 4/8 sheep in MED + ATI group and in 1/8 sheep in MV + ATI group.

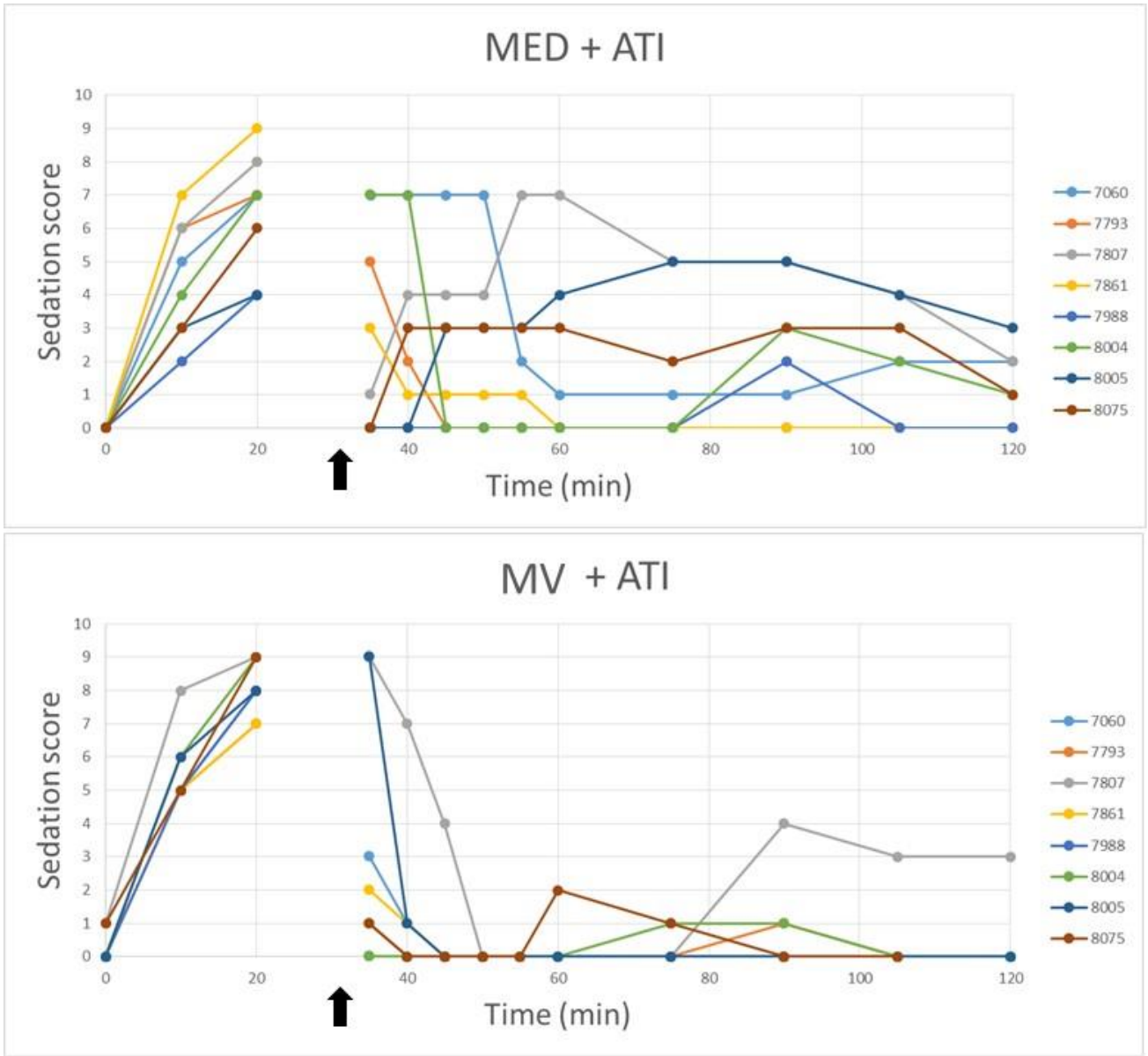

Figure 5. Sedation scores of eight sheep treated with medetomidine (30 µg/kg IM) (MED + ATI) or medetomidine (30 µg/kg IM) and vatinoxan (300 µg/kg IM) (MV + ATI) in same syringe. Sedation was reversed with atipamezole (15 µg/kg IM) after 30 minutes in both treatments (black arrow). Identification number of each sheep is presented on right.

5 DISCUSSION

As expected, vatinoxan alleviated some of the cardiopulmonary alterations caused by medetomidine. In the present study, HR was significantly higher with vatinoxan at 90 minutes after medetomidine administration and later. The significant difference in HR between treatments at 90 minutes and later might partly be explained by faster and more precise reverse with the presence of vatinoxan. After the reverse the sheep were more alert after MV + ATI and struggling to get out of the sling.

HR tended to decrease initially more rapidly with vatinoxan than without it. Similarly in dogs, a faster initial decrease of HR with vatinoxan (500 µg/kg IM) has been detected, when treated with medetomidine (20 µg/kg IM) and butorphanol (0.1 mg/kg IM) administered in the same syringe (Salla et al. 2014). After initial decrease, HR was significantly higher in dogs treated with vatinoxan compared to dogs without it (Salla et al. 2014). In sheep there are no previous studies about changes in pharmacokinetics of IM medetomidine caused by vatinoxan, but drug concentration data collected during the present study shows that presence of vatinoxan leads to faster absorption of medetomidine (Adam et al. 2018a). Faster absorption of medetomidine is a plausible explanation for initial decrease of HR in vatinoxan group.

In present study MAP was significantly higher at 10 minutes with the presence of vatinoxan compared to mere medetomidine. This is in contrast to Raekallio et al. (2010), who observed a significant early decrease of MAP with vatinoxan (250 µg/kg IV) in sheep sedated with dexmedetomidine (5 µg/kg IV). In their study MAP decreased with vatinoxan initially in few minutes to the level that was reached after 20 minutes with mere dexmedetomidine (Raekallio 2010). With mere dexmedetomidine there was a significant early increase in MAP (Raekallio et al. 2010). Similar results were reported by Adam et al. (2018b), who noted, that vatinoxan induced a dose-dependent initial decrease in MAP. Early decrease of MAP with vatinoxan was not observed in present study, conversely MAP increased significantly faster with vatinoxan than without it. Explanation could be the same as with initial decrease of HR: vatinoxan accelerates the absorption of medetomidine from the muscle tissue to blood (Adam et al. 2018).

Initial increase of MAP after mere medetomidine is considered to be a consequence of peripheral vasoconstrictive effect of medetomidine and the latter decrease to follow the centrally controlled relaxation and the sleep-like sedation induced by medetomidine after peripheral effect has faded (Kobinger 1978, Savola 1989, Maze & Tranquilli 1991). In present study, CO was measured and SVR thus calculated first time at 20 minutes after initial drug administration, when vatinoxan alleviated the increase of SVR. However, it is not known if there was an initial increase of SVR in MV+ATI before that, but it might be, as presence of vatinoxan did not attenuate the increase in MAP yet at 10 minutes after administration. Latter centrally controlled decrease in MAP was not seen in present study with or without vatinoxan, probably because the sedation was reversed with atipamezole before the change in MAP would have occurred (Celly et al. 1997). There was no significant difference between the treatments in MAP after atipamezole administration.

Lack of significant differences in CI in the present study with IM administration was in contrast with previous studies with IV administration in sheep (Raekallio et al. 2010) and dogs (Honkavaara et al. 2011, Salla et al. 2014) and with IM administration in dogs (Salla et al. 2014). However, in the present study CI tended to be higher with vatinoxan, but the difference was not significant. Early decrease of PaO_2 in with vatinoxan was also a surprising finding, but it is in line with early decrease of HR and increase of MAP and SVR. DO_2I was calculated first time at 20 minutes after initial drug injection, when it did not differ significantly between treatments, yet it is not known, if DO_2I decreased initially before that with PaO_2.

The effect of vatinoxan to the sedation in the present study was in line with the results reported in cats with IM dexmedetomidine (Honkavaara et al. 2017) and in dogs with IM medetomidine (Restitutti et al. 2017), in which vatinoxan shortened the onset time of clinical sedation, when administered simultaneously in same syringe with the sedative. The results of this study support the idea, that vatinoxan administered IM in the same syringe with medetomidine may not completely prevent the early cardiopulmonary effects of medetomidine but the variables return faster to the baseline level. This seems to be in line with previously reported results in dogs (Salla et al. 2014, Restitutti et al 2017).

According to our findings, vatinoxan is safe to use in sheep also, when the sedation is reversed with atipamezole. Cardiopulmonary variables did not differ significantly between treatments after the atipamezole injection, except for HR, which was higher with vatinoxan than without it. In conclusion, vatinoxan accelerates the onset of clinical sedation, alleviates the cardiopulmonary adverse effects of medetomidine after 10 minutes and relieves resedation after initial reversal induced by atipamezole with no other impact, when administered simultaneously with medetomidine.

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